Applications for Fluorescence Microscopy

Application Note

Basics

Fluorescence microscopy is an important optical tool within life sciences to monitor dynamic cellular processes in living specimens. A fluorescence microscope is basically a light microscope that uses the fluorescence light emanating from fluorescent dyes within the sample for imaging.

Fluorescent dyes are molecules that have the property to absorb light of certain wavelengths (mostly in the UV range) and to subsequently emit longer wavelengths in the visible range. Thus, the image from a fluorescence microscope is not created by the light used to illuminate the sample, but from the fluorescence emitted by different fluorescent dyes. Therefore, the optical components of a fluorescence microscope are optimized in order to separate the emission from the often overlapping excitation light.

The wide spectrum of target-specific fluorescent dyes available today allows one to selectively stain cellular components such as organelles, proteins, or even molecules. A key advantage of fluorescence microscopy is the ability to track the localization and dynamics of single or even multiple cellular components of interest within living systems by observing the location of the different fluorescent dyes. Thus, this imaging technique provides insights into specific aspects of often complex cellular functions.

The Principle of Fluorescence

Fluorescence is a process of luminescence and can be represented by the Jablonski diagram:

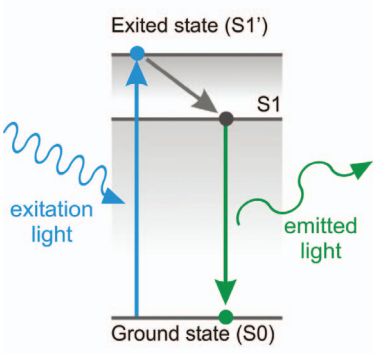

Figure 1.

1. When a fluorescent molecule (fluophore) absorbs light, its electrons become excited to a more energetic state (S1'). This process generates an excited state of the molecule.
2. Then, some internal conversions or vibrational relaxations (due to collisions with other molecules) of the absorbed energy occurs, putting the electron into a lower energy state (S1).
3. The electron returns to the ground state and the remaining energy is emitted as light. Since energy is lost prior to the emission, the wavelength of the emitted light is always longer than the absorbed light. Generally, the temporal delay between absorption and emission is on the order of nanoseconds so the two processes take place almost simultaneously.

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Characteristics of Fluophores

Each fluophore is characterized by its specific spectral properties. These spectral properties are directly related to the possible excited states of the fluophore. The possible excited states, in turn, depend on the energy levels to which the electrons of a fluophore can transit.

The excitation spectrum reflects how strongly a given fluophore absorbs the excitation light as a function of wavelength. The emission spectrum covers the range of emitted wavelengths. Both spectra can be determined empirically.

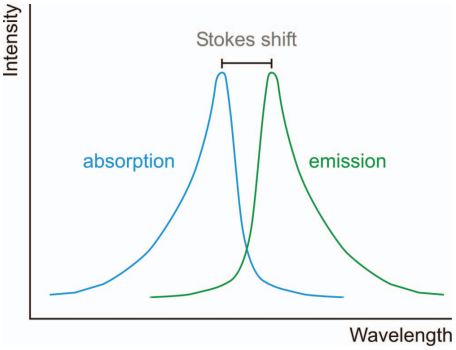

Figure 2. The Stokes shift — which often differs among different fluophores — is defined as the distance between the peak of the excitation spectrum and that of the emission spectrum.

There is a wide selection of fluorescent probes available that have been designed to bind to virtually any cellular targets.

Many fluophores can change their absorption and/or emission properties in response to certain parameter changes within their environment (for example, when being bound to intracellular molecules like calcium or hydrogen ions). Thus, using time-lapse fluorescent microscopy, the physiological state of cells and dynamic cellular signaling events can be visualized in living systems.

Some proteins are intrinsically fluorescent. A prominent example, the green fluorescent protein (GFP), is found in bioluminescent organisms like the jellyfish Aequorea victoria. Cell biologists utilize the variety of naturally fluorescent proteins in order to tag them to target proteins of interest by genetically fusing the DNAs of the proteins using appropriate methods. This technique provides a very powerful tool for localizing and trafficking proteins within living cells or studying the role of proteins in mediating intracellular signaling pathways.

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